EUSPORA ZEALANDICA ALLISON (APICOMPLEXA: SPOROZOA): A EUGREGARINE WITH A SPORE SIMILAR IN ULTRASTRUCTURE TO THAT OF SOME COCCIDIAN SPOROCYSTS

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ABSTRACT

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The spores of *Euspora zealandica* were examined with the electron microscope. They were found to be composed of an endospore, an exospore, and an epispore. The endospore is thick (230 nm) and bisected by a suture line. The exospore is thin (27 nm) and osmiophilic. The epispore is composed of a felt-like matrix which is folded into longitudinal ridges overlying the suture line and into terminal projections surrounding the poles. This spore structure is similar to the structure of the sporocysts of those coccidians where the sporozoite is released from the sporocyst by a simple rupture along a suture line.

KEYWORDS: Eugregarinida - spores - ultrastructure - Coccidia - sporocyst.

INTRODUCTION

The ultrastructure of the spores of a number of eugregarine parasites has been described (Abro 1976, Crespi *et al.* 1981, Porchet-Henneré & Fischer 1973, Prensier 1970a, Sanders & Poinar 1973, Sathananthan 1972) but no unifying structures have been recognised as yet, unlike the spores of their relatives, the coccidians. Amongst the coccidians two basic patterns of sporocyst ultrastructure have been shown depending on whether the sporozoites are released via the steida body or via a suture line. The wall of the sporocysts with a steida body tends to be made up of one layer of tightly bound membranes (Ferguson *et al.* 1978, Marchiondo *et al.* 1978, Roberts *et al.* 1979). The wall of the sporocysts with suture lines tends to comprise at least two layers: the inner one being thick, electron-dense and homogeneous, and the middle or outer layer being much thinner and osmiophilic (Box *et al.* 1980, Duszynski & Speer 1976, Ferguson *et al.* 1979, Lom 1971, Mehlhorn & Scholtyseck 1974, Odense & Logan 1976, Porchet-Henneré 1968, 1971, 1972, Porchet-Hennere & Richard 1969, 1971, Speer *et al.* 1973, 1976). In *Coelotropha durchoni* (Porchet-Henneré 1968, 1971) and opossum - passerine *Sarcocystis* sp. (Box *et al.* 1980), the thin osmiophilic layer is covered by an outer layer which is described by Porchet-Henneré (1968, 1971) as electron-lucent and by Box *et al.* (1980) as a matrix.

The ultrastructure of the spore of the eugregarine *Euspora zealandica* Allison 1969 was examined to determine any similarities with other gregarine spores. This paper describes the ultrastructure of the *E. zealandica* spore and compares it with previous descriptions of eugregarine spores and coccidian sporocysts.

MATERIALS AND METHODS

MICROSCOPY

Host larvae were killed in ether and dissected. Guts infected with intestinal eugre-

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garines were removed and the rectum was searched for gametocysts. Any gametocysts removed from the rectum were placed on microscope slides and the slides were sealed into moistened petri dishes with petroleum jelly. The gametocysts were allowed to develop in the petri dishes at room temperature (about 20°C).

E. zealandica spores for scanning electron microscope (SEM) examination were fixed (2% w/v paraformaldehyde, 2% v/v glutaraldehyde in 0.1 M phosphate buffer (PB); pH 7.2) for 3 h at room temperature, air dried, attached to SEM stubs with double-sided adhesive tape, sputter coated with gold and viewed with the SEM (ETEC/Siemens Autoscan) at 20 KV.

Spores for examination with the transmission electron microscope (TEM) were embedded in 3% w/v agar. The agar was fixed (6% glutaraldehyde in 0.1 M PB overnight), washed (0.1 M PB), post fixed (0.4% w/v osmium tetroxide in 0.1 M PB for 15 mins followed by 0.5% w/v potassium permanganate in 0.9% w/v sodium chloride for 1 h), washed (0.1 M PB), dehydrated in an ethanol series, embedded in Agar 100 resin, polymerised at 60°C for 48 h and sectioned. Sections were double stained with saturated uranyl acetate in 70% ethanol and 0.5% w/v lead citrate and viewed with the TEM (Hitachi IIA or Siemens Elmiskop 102) at 75 KV or 80 KV.

HOSTS

E. zealandica was obtained from the gut of third instar larvae of: *Costelytra zealandica* (Melolonthinae: Coleoptera), collected from Wendonside, Southland, N.Z., (45°46′ S, 168°45′ E) and from McCraes Flat, Otago, N.Z. (45°15′ S, 170°42′ E); and *Odontria striata* (Melolonthinae: Coleoptera) collected from Miller's Flat (45°41′ S, 169°29′ E) and from Herbert Forest (45°15′ S, 170°42′ E), Otago, New Zealand.

RESULTS

Gametocysts of *E. zealandica* from *C. zealandica* were reared to maturity. Dehiscence of the mature gametocyst occurred by simple rupture after one month.

When viewed in thin section with an electron microscope (Fig. 1), the spore appears elliptical with terminal projections, but when viewed with a light microscope (LM) the spore appears rectangular (Fig. 2). When measured with the LM the spores average 4.3 µm wide (range 4.1-4.6 µm) at the widest point and the average total length including projections is 11.0 µm (range 10.6-11.7 µm) and without them the length averages 7.4 µm (range 7.0-8.2 µm). Under the TEM the spore wall can be seen to be composed of three layers; an endospore and an exospore, making up the underlying elliptical shape, and an epispore, which is formed into the terminal projections and ridges on the surface of the spore.

The endospore of the mature spore is moderately electron-dense, homogeneous, about 230 nm thick, and is bisected by a suture line. In the developing spore the endospore is laminated, and the location of the suture lines is indicated by a number of striations crossing the endospore at right angles (Fig. 3). In more mature spores these striations are parallel to the suture walls. The suture follows a predetermined meridial line passing through each end and dividing the spore into two plates.

The exospore is osmiophilic and about 27 nm thick. As the endospore opens along its suture line, the corresponding parts of the exospore are progressively deformed. The opening of the suture line appears to be brought about by the curling inwards of the two plates. As the plates curl, the gap between the suture walls widens (Figs. 3-7), and the exospore is bent inwards (Figs. 5 & 6). In some micrographs the exospore can be seen to have split along the centre, parallel to the endospore (Figs. 5 & 6). In such cases the inner part of the exospore bends inwards with the endospore while the outer part bows outwards (Fig. 5). As the suture walls part, the exospore also fractures (Fig. 6). The presence of a split along the centre of part of the exospore immediately above the suture striations was also seen in an immature spore (Fig. 3), which suggests that the exospore is predisposed to splitting in this manner.

The epispore covers the surface of the spore and forms the longitudinal ridges and terminal projections (Fig. 1). It is composed of a trans-

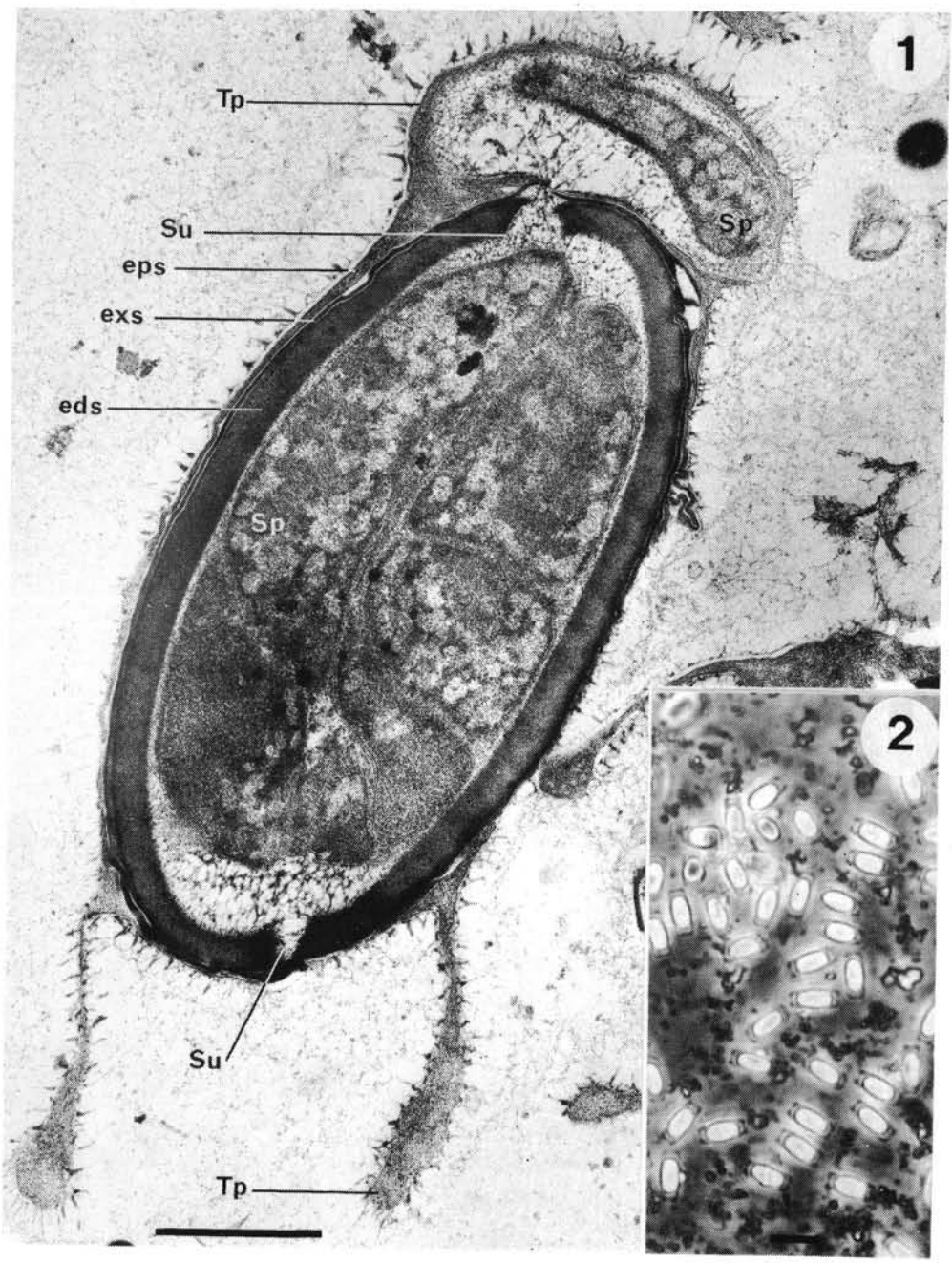
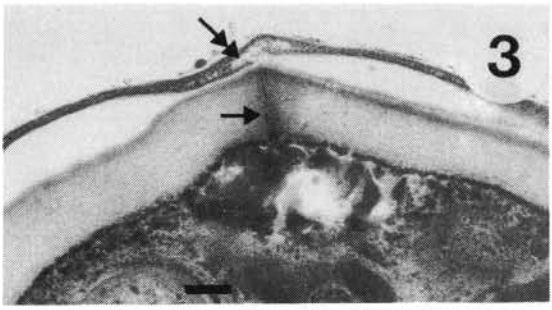


Figure 1. Ultrathin longitudinal section of a spore. The spore wall comprises, an endospore (eds), an exospore (exs), and an epispore (eps). The epispore is formed into the terminal projections (Tp), within which is a sporozoite (Sp). Other sporozoites are present inside the spore. The exospore and endospore give the spore its underlying elliptical shape. The endospore is interrupted by a suture line (Su) (bar = 1 μm).

Figure 2. A phase contrast micrograph of a wet mount of spores (bar = 10 μm).



Figures 3-7. Transmission electron micrographs of the spore wall of *Euspora zealandica* with suture lines at various stages of opening.

Figure 3. A closed suture line through the endospore of the spore wall of an immature spore. Striations (single arrow) are present in the suture walls. The exospore is split along its centre (double headed arrow) (bar = 100 nm).

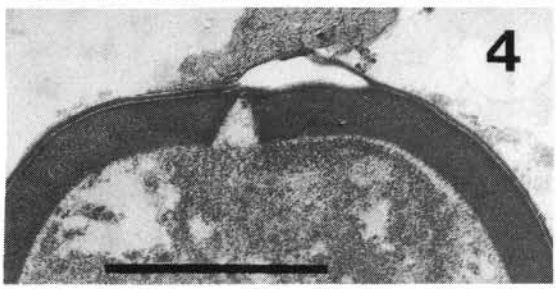


Figure 4. An open suture line through the endospore (bar = 1 μm).

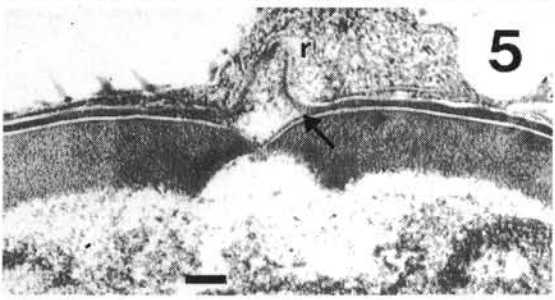


Figure 5. A wide open suture line. The endospore has curled inwards and the exospore has split along its centre (single arrow). In this spore a longitudinal ridge (r) formed by the epispore is immediately above the suture line (bar = 100 nm).

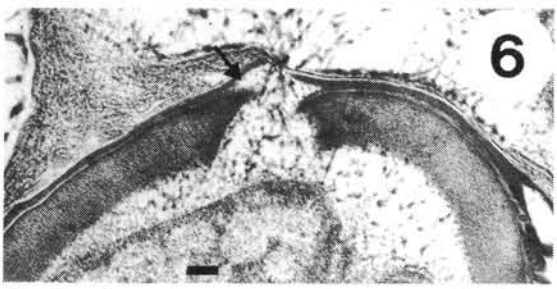


Figure 6. The endospore has parted at the suture line and the exospore and epispore have parted above the suture line. The exospore has also split along its centre (single arrow) (bar = 100 nm).

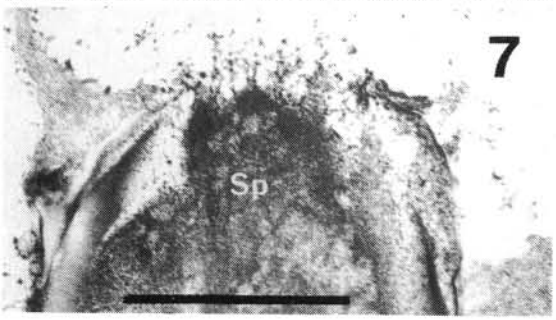


Figure 7. The spore wall is open and a sporozoite (sp) can be seen in the opening (bar = 1 μm).

Figures 8 & 9. Scanning electron micrographs of the spore surface of *E. zealandica*.

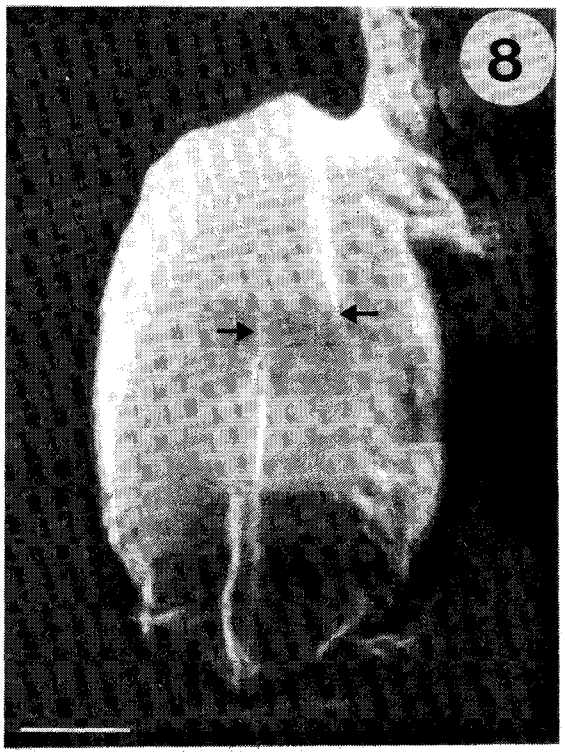


Figure 8. A longitudinal fold on the surface of this spore is in two parts which slightly overlap (single arrow) (bar = 1 µm).

Figure 9. The longitudinal fold is continuous along the length of this spore. The terminal projections are represented by the small ridge that encircles the end of the spore (bar = 1 µm).

parent outer membrane, with conical adhesions, overlying a felt-like matrix. The transparent membrane is about 20 nm thick and the conical adhesions measure up to 240 nm in length. The matrix component is made up of tubules or cavities lying at random and it varies in thickness from about 25 nm to 670 nm under the longitudinal ridges.

SEM micrographs show a predominantly smooth spore surface with a longitudinal ridge running the length of the spore and either a plain rounded end (Fig. 8) or an end encircled by a ridge (Fig. 9). The longitudinal ridges seen in the SEM correspond to the thickened areas of the epispore seen in sections of the spore. In a number of specimens the longitudinal ridge is in two parts which slightly overlap in the middle (Fig. 8), and some spores viewed in the SEM apparently had no longitudinal ridges. In a number of sections the longitudinal ridges were found directly overlying the suture lines but the association of the two was not confirmed in every case. No projections could be distinguished in the SEM micrographs but the ridges observed encircling the ends of the spores were located about where the terminal projections would be expected to arise based on LM and TEM observations. In places the terminal projections were very thin (37 nm) (Fig. 1), and they probably collapsed during preparation for the SEM.

DISCUSSION

E. zealandica was first described from the larvae of the melolonthid, *C. zealandica*, in Canterbury, New Zealand (Allison 1969) and subsequently recorded in larvae of the dynastid, *Oryctes* sp. from Papua New Guinea (Théodoridès *et al.* 1972). It is therefore not surprising to find it in other areas of New Zealand (Otago and Southland) and in another melolonthid, *O. striata*.

At present, the genus *Euspora* is described as having spores which are a prismatic shape. However, present data show the spores of *E. zealandica* to be ellipsodial and covered with an epispore. This description does not appear to be at variance with the type species of the genus, *E. fallax*, as diagrams in Geus (1969) show that it too has an underlying ellipsoidal spore that is covered by an epispore. The epispore of *E. fallax* gives it its prismatic shape. Thus it may be more accurate to describe the spores of the genus as ellipsoidal with an epispore rather than as prismatic.

The structure of the spore of *E. zealandica* differs from that of all previously described spores of the eugregarines or neogregarines (Åbro 1976, Crespi *et al.* 1981, Porchet-Henneré & Fischer 1973, Prensier 1970 a, b, Sanders & Poinar 1973, Sathananthan 1972, Vavra & McLaughlin 1970, Zizka 1977), but in a number of respects it is similar to the ultrastructure of the sporocyst walls of those coccidian species where dehiscence occurs via a suture line (Box *et al.* 1980). Both *E. zealandica* and the coccidians have a thick, electron-dense, homogeneous endospore that is bisected by suture lines, and an adjacent, much thinner, osmiophilic exospore. As is the case with coccidians, the suture lines seen in *E. zealandica* divide the spore into plates, and at dehiscence the plates curl inwards, dragging in the outer layer, which finally ruptures. However, unlike the coccidians, there are no prominent lips or an interposing strip in *E. zealandica*.

The SEM reveals a smooth surface on the spores of the gregarines *Actinocephalus acanthaclisis* (Marques & Ormières 1978) and *Mattesia dispora* (Zizka 1978), but no gregarine spores have been described with ridges similar to those seen in *E. zealandica*. However, surfaces of the sporocysts of the coccidians, *Toxoplasma gondii* (Ferguson *et al.* 1982) and *Sarcocystis* sp. (Box *et al.* 1980) are mainly smooth but have ridges that overlie the suture lines, somewhat similar to those noted for *E. zealandica*.

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